Molecular Biology

MEMBRANE PROTEIN CRYSTALLIZATION IN THE CUBIC PHASE OF A LIPID SOLUTION

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A comprehensive understanding of the biology of membrane proteins requires knowledge of the structural basis for observed functions. Current methods in x-ray crystallography can return atomic resolution structures of membrane proteins if three-dimensional crystals can be generated. The amphiphilic nature of membrane proteins, however, makes crystallization of these proteins especially difficult, and these experiments have historically utilized detergents and aqueous solutions to facilitate solubilization, purification, and crystallization by vapor diffusion. Such methods have not proven universally applicable and, when successful, have not necessarily provided optimal crystals for x-ray diffraction studies. As an example, the photosynthetic reaction center of *Rhodobacter sphaeroides* has frequently been crystallized by conventional vapor diffusion methods. Despite this, all such crystals have defied freezing, thereby limiting their analysis at third generation beam lines. In light of such restrictions, the development of crystallization techniques using the cubic phase of a lipid solution is a welcome advance available to membrane proteins (detergent-solubilized and lipid-bound polypeptides) as well as soluble proteins. Furthermore, this method of crystallization gains tremendous potential utility in its provision of both cryo- and physical protection to protein crystals by the relatively rugged lipid cubic phase environment. In addition, crystallization in this hydrophobic setting may offer structures of more natively folded membrane proteins. Using monoolein in a 60% w/v, 17% (v/v) jeffamine M600, 750 mM Hepes pH 7.5, 0.05 mg/uL ammonium sulfate, and 3-9 mg/mL protein as final concentrations in a 20 uL trial volume, we have repeated previous cubic phase crystallization of *R. sphaeroides* reaction centers, yielding single crystals. The largest of these crystals measured ~400 µm x 250 µm x an undetermined third dimension. This crystal was of trigonal bipyramidal shape and was much larger than crystals previously reported to have been produced by lipid cubic phase techniques. Attempts to prepare this crystal for x-ray diffraction were unsuccessful; however, additional trials to produce similar crystals have been initiated. Numerous additional trials have commenced with various other proteins, precipitants, buffers, and salts in an attempt to produce crystals of these proteins and examine the nature of the lipid cubic phase. In this report we discuss the nature of our cubic phase crystallization trials and provide a more generalized comparison of these techniques to conventional crystallization methodology.